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Original Article

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LncRNA FPASL suppresses fibroblast proliferation through its DNA methylation via DNMT3b in hypertrophic scar

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Abstract

Long noncoding RNAs (lncRNAs) are increasingly being implicated as key regulators of cell proliferation, apoptosis, and differentiation. However, the molecular mechanisms of specific lncRNAs in the context of hypertrophic scar remain largely unclear. Here, we find that the lncRNA FPASL (fibroblast proliferation-associated LncRNA) is downregulated in HS, and FPASL reduces fibroblast proliferation and colony formation and blocks cell cycle progression. Using GO annotation enrichment analysis along with AZC (a specific inhibitor of DNA methylation), we identify that DNA methylation is responsible for downregulating FPASL in hypertrophic scar. Subsequent studies demonstrate that high expression of DNMT3b inhibits FPASL expression in HS. Mechanistic study reveals a significant increase in fibroblast proliferation after transfection with LNA-FPASL, which is further inhibited by knockdown of *DNMT3b*. Thus, our study reveals that DNMT3b mediates hypermethylation of the lncRNA FPASL promoter and the downregulation of lncRNA FPASL promotes fibroblast proliferation in hypertrophic scar.

Key words lncRNA FPASL, DNA methylation, fibroblast, hypertrophic scar

Introduction

Millions of people annually develop scars in many life situations, such as skin burns, surgical incisions, physical trauma, vaccinations and even insect bites [1,2]. At the end of normal cutaneous wound healing, fibroblast proliferation slows down and cells stop synthesizing more extracellular matrix (ECM) [3,4]. Excessive fibroblast proliferation results in the accumulation of ECM and triggers the formation of hypertrophic scar [5]. Excessive proliferation of fibroblasts is therefore a hallmark of hypertrophic scar. Recently, an increasing body of evidence has suggested that long noncoding RNAs (lncRNAs) have been implicated in fibroblast proliferation of hypertrophic scar [6,7]. LncRNAs are a heterogeneous group of noncoding transcripts with lengths greater than 200 nucleotides [8]. Changes in their expression and function bring about the pathogenesis of a series of diseases [9,10]. However, the molecular mechanisms of lncRNAs in hypertrophic scar remain poorly understood and warrant further study.

Epigenetic regulation is one of the mechanisms regulating lncRNA expression and tissue specificity, particularly DNA methylation alteration [11,12]. Aberrant DNA hypermethylation at gene promoter regions catalyzed by DNA methyltransferases (DNMTs) can repress gene expression, and many studies indicated the existence of links between DNA methylation and diseases [13]. For example, upregulation of lncRNA SNHG12 caused by DNA methylation promotes temozolomide resistance in glioblastoma [14]. The essential role for DNA methylation levels in hypertrophic scar has

recently been revealed [15]. PTEN hypomethylation could inhibit fibroblast phenotypic transformation, and DNMT1 inhibition by a low dose of DNA demethylating agent (5-aza-2-deoxycytidine) has been shown to inhibit proliferation and promote apoptosis of hypertrophic scar fibroblasts [15], indicating that the generation of hypertrophic scar may be related to methylation of certain genes. However, the molecular mechanisms by which DNMTs regulate hypertrophic scar fibroblasts remain largely elusive.

In the present study, we investigated the role of lncRNA FPASL (fibroblast proliferation-associated LncRNA) in fibroblast proliferation of hypertrophic scar and the effect of epigenetic regulation on its abnormal expression. Our results showed that DNMT3b-mediated FPASL promoter hypermethylation leads to downregulation of FPASL expression in hypertrophic scar. FPASL inhibits fibroblast proliferation and the G1/S cell cycle transition.

Materials and Methods

Patients and specimens

Eighteen hypertrophic scar (HS) tissues and their corresponding adjacent normal (NS) tissues were obtained from 18 different patients who were admitted to the Affiliated Hospital of Ningxia Medical University (Yinchuan, China) for scar removal. A diagnosis of a hypertrophic scar was confirmed by routine pathological examination. The collected skin samples were divided into three parts: one was used for the isolation and culture of fibroblasts, one was used for the preparation of protein, and the last one was immediately frozen in liquid nitrogen for the preparation of total RNA. This study was approved by the Medical Ethics Committee of Ningxia Medical University (Yinchuan, China). Patients admitted to our hospital for scar removal were provided information about the purpose of the study, and written informed consent was obtained from each participant.

Cell culture

HS and NS were taken from three patients who had not been treated for burns before surgery. The samples were pruned to remove excess adipose tissue, rinsed three times with phosphate buffer saline (PBS) containing gentamicin (Biotopped, Beijing, China) and penicillin-streptomycin solution (Solarbio, Beijing, China), cut into small pieces, and then incubated in DPBS (HyClone, Logan, USA) containing 0.125% TE (Solarbio) at 4°C overnight. The next day, the solution was removed and the tissues were minced and incubated in DMEM-F12 medium (HyClone) containing 0.1% collagenase type I (Solarbio) at 37°C for 6–8 h, and then filtered through a 200-mesh screen. The isolated cells were cultured in DMEM-F12 medium containing 10% fetal calf serum (Biological Industries, Kibbutz Beit-Haemek, Israel) and 1% penicillin-streptomycin solution at 37°C in an atmosphere of 5% CO_2. The cell strains were maintained and analyzed from passage 3 to passage 6.

RNA sequencing and gene ontology (GO) enrichment analysis

Sequencing was performed on the Illumina HiSeq 2000 platform (Illumina, San Diego, USA), and 150-bp paired-end reads were obtained. mRNA and noncoding RNA were randomly broken into short fragments of 200–500 bp in length. The short fragments were used as templates to synthesize the first strand cDNA with 6-base random primers, and dUTP was used instead of dTTP to synthesize the second strand cDNA. After purification of the synthesized double-stranded cDNA, terminal repair and A-tail addition were performed, and the sequencing linker was ligated. Furthermore, uracil-N-glycosylase was added to degrade the second strand. The fragment size was selected by agarose gel electrophoresis followed by PCR amplification. The final sequenced library was sequenced on the Illumina sequencing platform (Illumina).

GO enrichment analysis of lncRNAs was performed using the R language version 3.4.4 clusterProfiler, org.Hs.eg.db genome-wide annotation, the pathview R package, the top GO package, and the enrich plot package to analyze enrichment status. The ggplot2 package was used to create graphics in R. An FDR < 0.05 was used to compare the results. The GO terms included the following three criteria: molecular function (MF), cellular component (CC), and biological process (BP).

Lentivirus and LNA GapmeR transfection

FPASL overexpression lentivirus was purchased from Sheweisi (Tianjin, China), DNMT3b knockdown lentivirus was purchased from Genechem (Shanghai, China), the siRNA sequence of DNMT3b was 5′-CTCAAGACAAATTGCTATA-3′, and the siRNA sequence of shRNA-NC was 5′-TTCTCCGAACGTGTCACGT-3′. FPASL LNA GapmeRs were purchased from Qiagen (Carlsbad, USA), and the LNA GapmeRs sequences of FPASL were as follows: LNA-NC: 5′-GCTCCCTTCAATCCAA-3′; LNA-FPASL 1: 5′-AGAACGTTACAGTGAT-3′; LNA-FPASL 2: 5′-GGTAGTTAGGTGG TAT-3′; and LNA-FPASL 3: 5′-GCAGACCAAGAGTGAG-3′. For transient transfections, Lipofectamine 2000 reagent (Invitrogen, Carlsbad, USA) was used according to the manufacturer's instructions. The cells were collected after transfection with LNA GapmeRs for 48 h. The infection efficiency was validated by qRT-PCR. To generate stable *FPASL*- or *DNMT3b*-knockdown cells, FPASL- or DNMT3b-knockdown ORF was cloned into the lentiviral vector. Lentivirus with FPASL or DNMT3b knockdown was generated and used to infect fibroblasts. After 72 h of infection, infected cells were cultured in DMEM-F12 containing 4.5 μg/mL puromycin to select cells with stable FPASL or DNMT3b knockdown.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated and reverse transcribed into cDNA using M-MLV Reverse Transcriptase (TaKaRa, Tokyo, Japan). Then, a real-time PCR assay was performed using a SYBR Prime Script PLUS RT-RNA PCR kit (TaKaRa). The PCR conditions were 95°C for 30 s, 95°C for 5 s, and 61°C for 34 s, and the process was repeated 40 times. The relative expression was analyzed using the $2^{-\Delta\Delta Ct}$ method. *GAPDH* was used as an internal reference. The primer sequences are shown in Table 1.

Western blot analysis

Total protein was isolated using RIPA buffer (Beyotime Biotechnology, Shanghai, China) containing a protease inhibitor cocktail. Protein extracts were separated by 8%–10% SD-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were subsequently blocked in 5% defatted milk and incubated with primary antibodies overnight at 4°C, including anti-DNMT1 antibody (GeneTex, San Antonio, USA), anti-DNMT3a antibody (Abcam, Cambridge, UK), and anti-DNMT3b antibody (Proteintech, Chicago, USA). The membranes were then incubated with a horseradish peroxidase (HRP)-conjugated secondary

Table 1. Sequences of primers used in this study

Gene	Primer sequence (5′→3′)	
DNMT1	Forward	CGGCAGACCATCAGGCATTCTAC
	Reverse	CACACCTCACAGACGCCACATC
DNMT3a	Forward	GCAAGGAGGAGCGCCAAGAG
	Reverse	GCTCACTCCGCTTCTCCAAGTC
DNMT3b	Forward	GCCACCTTCAATAAGCTCGTCTCC
	Reverse	AGCTGGTCCTCCAATGAGTCTCC
GAPDH	Forward	ATTCCACCCATGGCAAATTCC
	Reverse	GACTCCACGACGTACTCAGC
FPASL	Forward	TACCGTCTCTTGGAAGGCAGTCTG
	Reverse	GCCTCGGTCTCCCAAAGTGTTG

antibody (ABclonal, Wuhan, China) for 2 h at room temperature. Finally, the antigen-antibody reaction was visualized using ECL chemiluminescent agents (Thermo Scientific, Waltham, USA).

MassARRAY quantitative methylation analysis

For every cleaved CpG site, the intensity of a pair of mass signals, one representing methylated DNA and/or the other representing unmethylated DNA, was recorded and analyzed using the MassARRAY EpiTYPER software (https://china.agenabio.com/). The relative methylation status was estimated by dividing the peak intensity, or area of the methylated DNA, by the sum of the intensities or areas of the methylated and unmethylated components. The ratios between methylated and unmethylated DNA were obtained as the quantity of methylation for further analysis.

Cell counting kit-8 (CCK-8) assay

The fibroblasts were seeded in 96-well plates to evaluate proliferation ability by CCK-8 assay. Cells were then cultured for 24 h before the addition of 10 μL of CCK-8 solution (5 mg/mL; APExBIO, Houston, USA) to the culture medium in each well. The absorbance was measured at 450 nm with a max microplate reader (Thermo Fisher Scientific).

Colony formation assay

Fibroblasts were seeded at a density of 300 cells/well in 24-well plates and cultured for 10 days to form cell colonies. Then, fibroblasts were washed twice with PBS and fixed with 4% polyoxymethylene for 15 min. Fixed cell colonies were stained with Giemsa for 10 min and photographed.

Flow cytometric analysis

For cell cycle analysis, fibroblasts were seeded into 6-well plates at a density of 2×10^5–1×10^6 cells per well. After treatment, cells were harvested, washed with PBS, and incubated with 1 mL DNA staining solution (Lianke, Hangzhou, China) and 10 μL permeabilization solution (Lianke) for 0.5 h at 37°C. The distribution of cell cycle phases was determined with an ACEA NovoExpress Flow Cytometer (Agilent, Santa Clara, USA). The percentages of cells in the G1, S, and G2 phases were calculated.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 software, and data were expressed as the mean ± SD. Statistical comparisons were made by one-way analysis of variance (ANOVA), and $P < 0.05$ was considered statistically significant.

Results

LncRNA FPASL suppresses fibroblast proliferation in hypertrophic scar

Previous studies have demonstrated that lncRNA expression profiles are significantly altered in various diseases [16] To determine the functions and mechanisms of lncRNAs in hypertrophic scar, a lncRNA microarray was used to identify differentially expressed lncRNAs in hypertrophic scar (HS) tissues and corresponding adjacent normal (NS) tissues. As expected, a variety of lncRNAs were identified to be aberrantly expressed in HS (Figure 1A). We found that lncRNA FPASL was remarkably decreased in HS. Then, we performed qRT-PCR analysis of 18 paired HS and NS samples (Figure 1B). FPASL expression was significantly lower in the HS group than in the NS group, which is consistent with the lncRNA microarray data (Figure 1A). Additionally, FPASL expression was decreased in primary fibroblasts from hypertrophic scar tissues (HSFBs; Figure 1C), indicating that FPASL is involved in the progression of hypertrophic scar. Moreover, it is well known that rapid proliferation contributes to the fast progression of hypertrophic scar. As we found that FPASL is expressed at low levels in HS tissue as well as primary fibroblasts, we further wondered whether FPASL is involved in the proliferation of fibroblasts. We constructed lentiviruses, and locked nucleic acid (LNA) GapmeRs for FPASL and FPASL expression were detected by qRT-PCR (Figure 1D). Among the FPASL LNA GapmeRs, LNA-FPASL-2 was selected for subsequent experiments because it exhibited the strongest knockdown efficiency. CCK-8 assay results showed that cell viability was obviously increased after knockdown of *FPASL* and decreased after FPASL overexpression (Figure 1E). Colony formation assays showed that the number of cell colonies in fibroblasts transfected with LNA-FPASL was significantly larger, while it was clearly smaller in fibroblasts infected with OE-FPASL (Figure 1F). We further assessed the effect of FPASL on the cell cycle. The results showed that overexpression of FPASL arrested cells in the G0/G1 phase and reduced the number of cells entering the S and G2/M phases, while knockdown of *FPASL* promoted the transition from the G0/G1 phase to S and G2/M phases (Figure 1G). These data indicated that FPASL suppresses fibroblast proliferation and cell cycle progression in hypertrophic scar.

DNA methylation regulates the expression of lncRNA FPASL in hypertrophic scar

GO annotation enrichment analysis of aberrantly expressed mRNAs was performed to better understand the biological processes between HS and NS. Biological process (BP) was significantly enriched for extracellular matrix organization, collagen catabolic process and collagen fibril organization. Significant cellular component (CC) terms revealed enrichment in proteinaceous extracellular matrix, collagen trimer and cytoskeleton. The three molecular functions (MFs) identified were extracellular matrix structural constituent, collagen binding and metalloendopeptidase activity (Figure 2A). The interaction between mRNA and lncRNA significantly affects the progression of many diseases, and the molecular and cellular characteristics of lncRNAs are under distinct regulatory regimes that are different from physiological conditions. To explore the mechanisms underlying the downregulation of FPASL, the UCSC Genome Browser was used. *FPASL* is located on

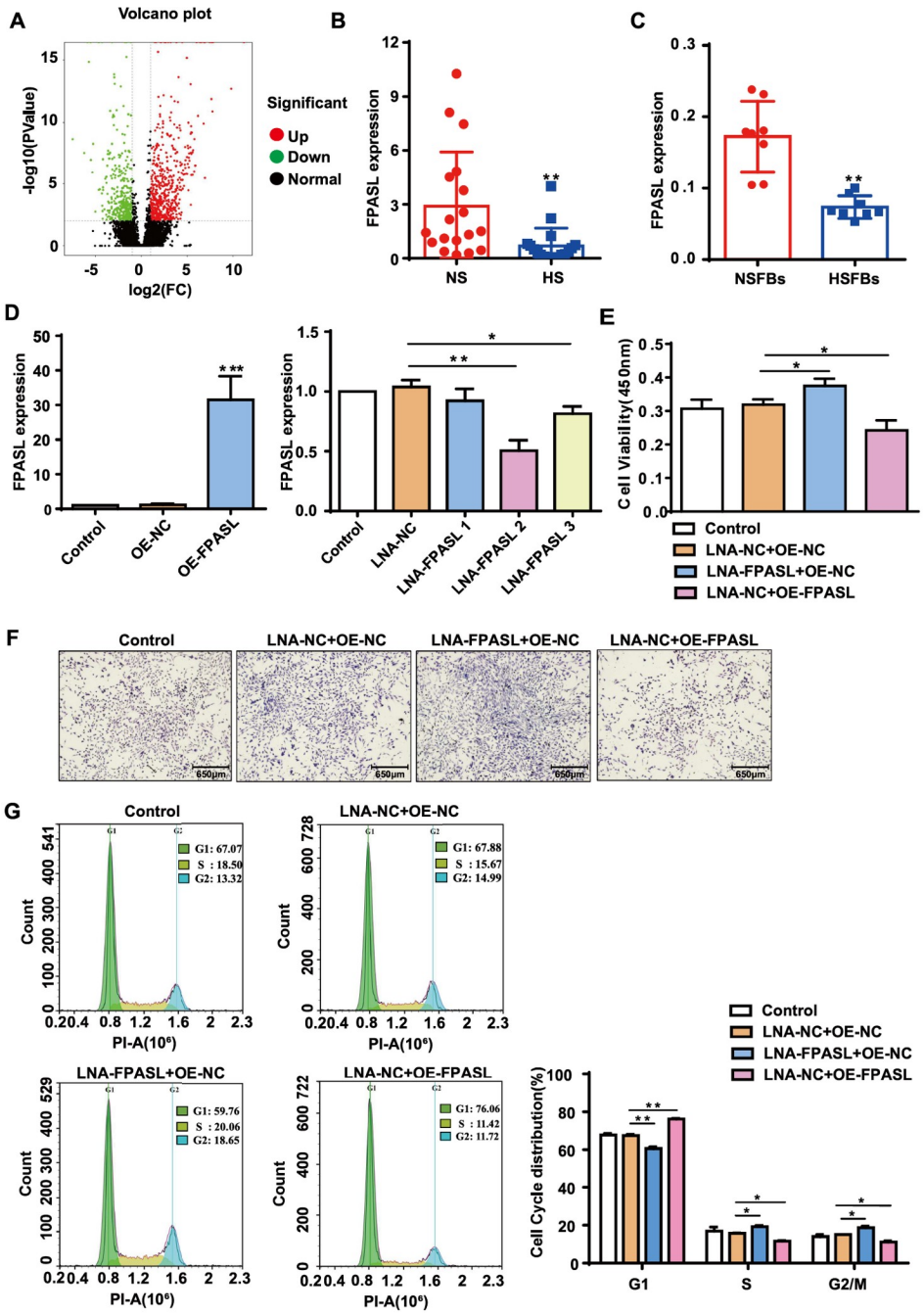

Figure 1. lncRNA FPASL affects fibroblast proliferation in hypertrophic scar (A) Volcano plots of differentially expressed lncRNAs in hypertrophic scar (HS) tissues and the matched adjacent normal (NS) tissues generated from the lncRNA microarray. Red dots indicate significant upregulation of genes, and green dots indicate significant downregulation of genes. Black dots represent no difference. (B) qRT-PCR was used to detect the expression of *FPASL* in 18 paired HS and NS tissues. (C) qRT-PCR was used to detect the expression levels of *FPASL* in primary fibroblasts from hypertrophic scar tissues (HSFBs) and matched adjacent normal tissues (NSFBs). (D) Efficacy of stable FPASL overexpression or knockdown in fibroblasts. (E) Cell viability of fibroblasts detected by CCK-8 assay after FPASL overexpression or knockdown for 48 h. (F) The number of colonies was measured by colony formation assay after FPASL overexpression or knockdown. Scale bar: 650 μm. (G) The cell cycle was verified by flow cytometric analysis in fibroblasts. $^{*}P < 0.05$, $^{**}P < 0.01$.

chromosome 7 (q11,23) and has an abundance of GC. Two potential CpG islands were identified by using the MethPrimer software: island 1: —2723 bp ~ —2328 bp; and island 2: —607 bp ~ —164 bp at the *FPASL* promoter relative to the TSS (Figure 2B), suggesting a potential relationship between FPASL expression and DNA methylation. To determine whether the downregulation of FPASL is resulted from its promoter hypermethylation, we detected FPASL expression by qRT-PCR analysis after primary fibroblasts were treated with AZC, a specific inhibitor of DNA methylation, and the results showed an increased expression of FPASL in fibroblasts treated with AZC (Figure 2C), indicating that downregulation of FPASL in hypertrophic scar may be associated with the level of DNA

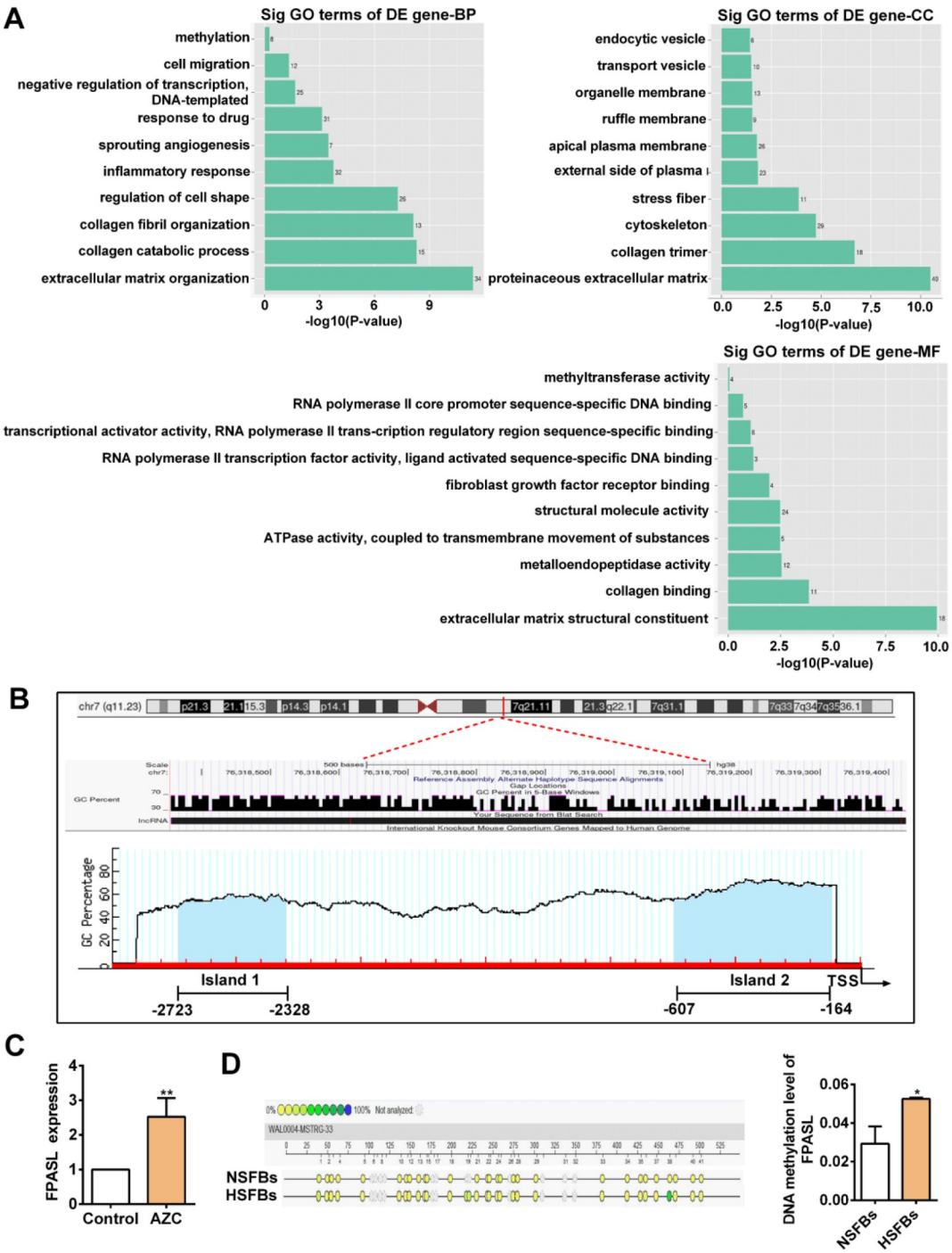

Figure 2. DNA methylation is involved in the downregulation of lncRNA FPASL in hypertrophic scar (A) GO annotation enrichment analysis of differentially expressed mRNAs in HS and NS. GO analysis included biological process (BP), cellular component (CC), and molecular function (MF). The count represents the number of genes in each pathway. (B) Schematic representation of the gene locus and CpG islands of FPASL. The gene locus and relative %GC content of FPASL were identified by the UCSC genome browser https://genome.ucsc.edu/cgi-bin/hgGateway?redirect=manual&source=genome.ucsc.edu. Meth Primer database http://www.urogene.org/methprimer2/ analysis of the CpG sites of the FPASL promoter ranging from –3000 to +1 bp. (C) qRT-PCR analysis was used to detect *FPASL* expression in fibroblasts treated with AZC for 24 h. (D) Mass array analysis was performed to examine the DNA methylation level of CpG islands at the promoter of FPASL in NSFBs and HSFBs. $*P<0.05$, $**P<0.01$.

methylation at its promoter. According to the analysis for the two islands, there is a higher percentage of GC on island 2. Thus, a further Mass Array analysis was used to detect the DNA methylation level of island 2 of the FPASL promoter, and the results showed an increased DNA methylation of island 2 in the FPASL promoter in HSFBs compared to that in NSFBs (Figure 2D), indicating that DNA methylation is responsible for the downregulation of FPASL in hypertrophic scar.

DNMT3b is a key enzyme in the downregulation of lncRNA FPASL in hypertrophic scar

To confirm the above findings, we detected the expression levels of DNA methyltransferases (DNMTs) in NS/HS tissues and NSFBs/HSFBs. As shown in Figure 3A, DNMT3b expression was significantly higher in HS than in NS, and DNMT3a was lower in HS than in NS. Similar results were obtained in NSFBs/HSFBs (Figure 3B), and DNMT1 also tended to be higher in HS/HSFBs than in NS/NSFBs, although the difference was not statistically significant (Figure 3A,B), implying that DNMT3b may be responsible for the downregulation of FPASL in the progression of hypertrophic scar. Then, we knocked down *DNMT3b* to determine the impact of DNMT3b on FPASL expression (Figure 3C). We found that FPASL expression was significantly increased after fibroblasts were infected with LV-sh-DNMT3b (Figure 3D). Moreover, *DNMT3b* knockdown led to decreased methylation level in the FPASL promoter (Figure 3E). These results suggested that DNMT3b is involved in the epigenetic regulation process of the FPASL promoter in hypertrophic scar.

DNA hypermethylation of lncRNA FPASL via DNMT3b contributes to fibroblast proliferation in hypertrophic scar

After identifying DNMT3b as a regulator of FPASL downregulation, we next analyzed whether DNMT3b mediates FPASL-regulated fibroblast proliferation. The CCK-8 assay results demonstrated that knockdown of *DNMT3b* inhibited LNA-FPASL-reduced fibroblast proliferation (Figure 4A). Colony formation assays showed that knockdown of *DNMT3b* obviously decreased the number of cell colonies in fibroblasts transfected with LNA-FPASL (Figure 4B). In addition, we observed that FPASL downregulation promoted G1/S cell cycle transition, and this effect was markedly abolished by *DNMT3b* knockdown (Figure 4C). Taken together, these data indicated that DNMT3b functions in the transcriptional inhibition of FPASL, which leads to fibroblast proliferation in hypertrophic scar.

Discussion

Hypertrophic scar causes itching, pain and even major joint dysfunction and troubles many patients [17]. However, we do not

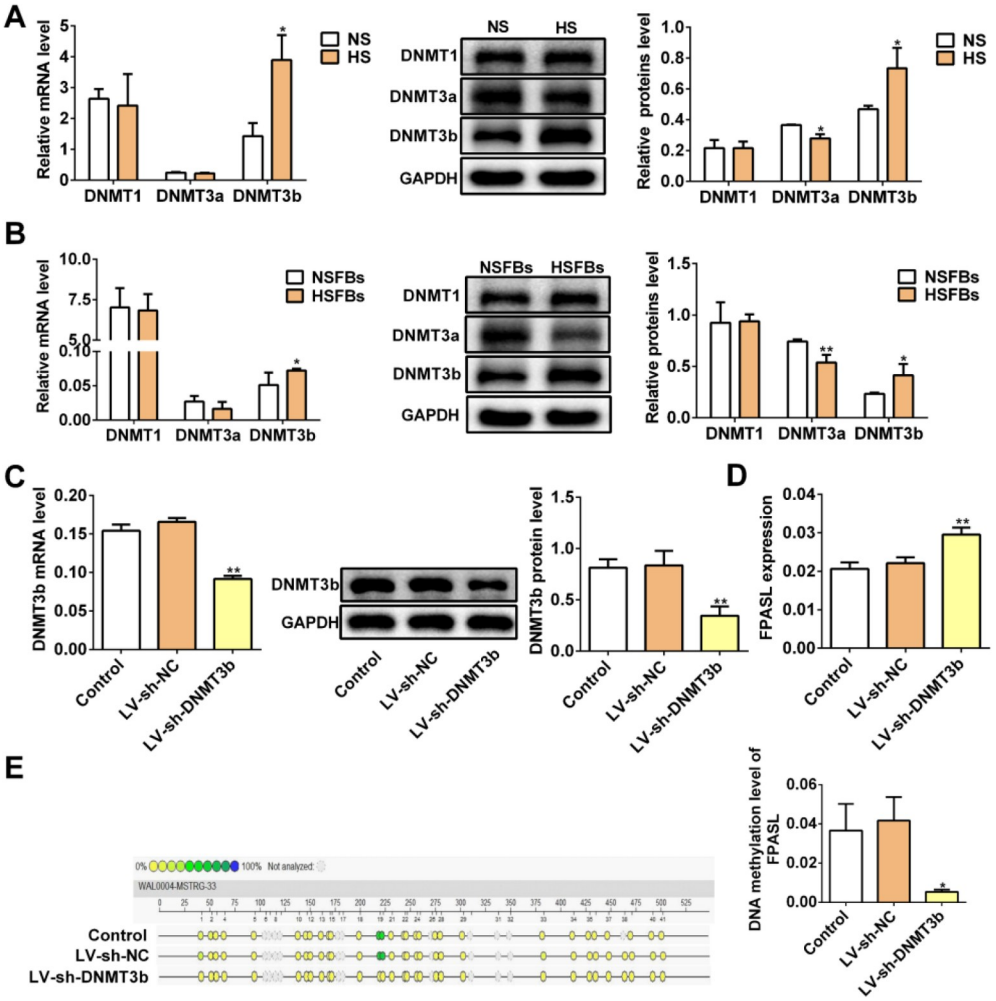

Figure 3. DNMT3b suppresses the expression of lncRNA FPASL in hypertrophic scar (A) DNA methyltransferase (DNMT) profiles in NS and HS tissues identified by qRT-PCR and western blot analysis. (B) DNMT profiles in NSFBs and HSFBs identified by qRT-PCR and western blot analysis. (C) DNMT3b expression was determined by qRT-PCR and western blot analysis after fibroblasts were transfected with LV-sh-DNMT3b or LV-NC. (D) qRT-PCR analysis of *FPASL* expression after *DNMT3b* knockdown. (E) Mass array analysis was used to examine the DNA methylation level of FPASL after *DNMT3b* knockdown. $^{*}P < 0.05$, $^{**}P < 0.01$.

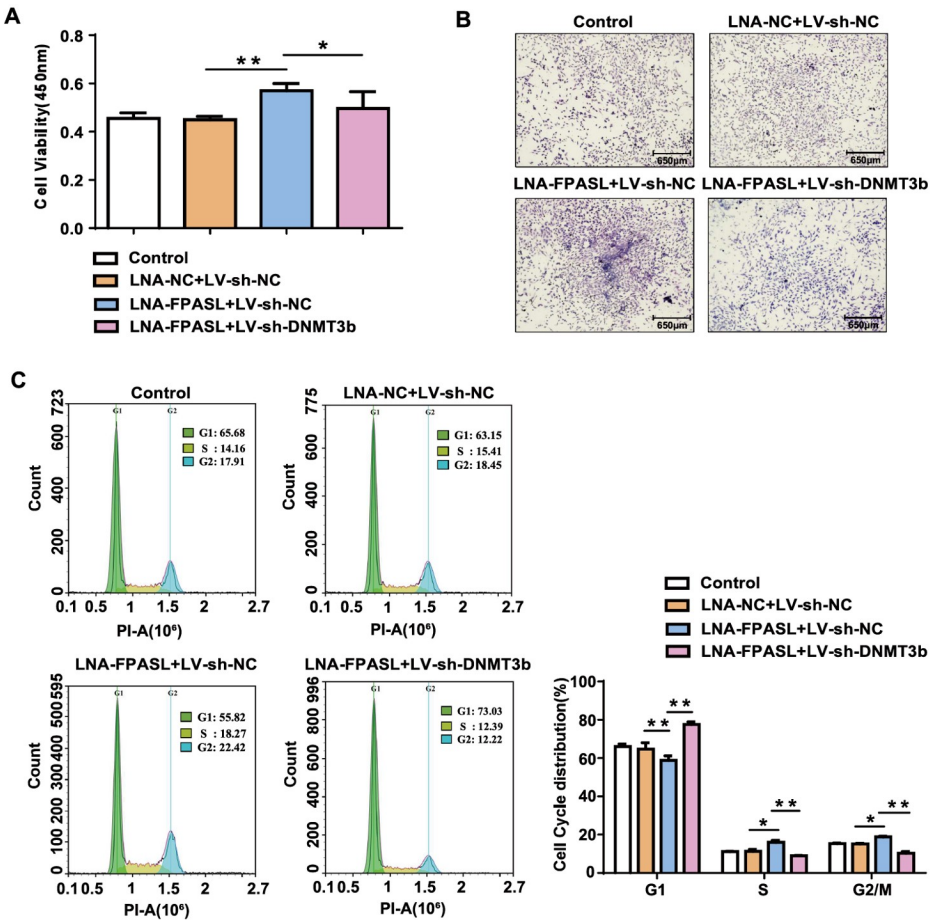

Figure 4. Downregulation of FPASL facilitates fibroblast proliferation via DNA methylation of its promoter in hypertrophic scar (A) The effect of *DNMT3b* knockdown on the viability of fibroblasts treated with LNA-FPASL, as detected by CCK-8 assay. (B) The effect of *DNMT3b* knockdown on the number of colonies of fibroblasts treated with LNA-FPASL was measured by colony formation assay. Scale bar: 650 μm. (C) The effect of LV-sh-DNMT3b on the cell cycle was verified by flow cytometric analysis in fibroblasts treated with FPASL. $^{*}P<0.05$, $^{**}P<0.01$.

have efficient ways to cure this disease because the mechanism of HS is still unclear. In recent years, the roles of lncRNAs in various diseases have been extensively studied [18–20]. It was shown that some lncRNAs increased the processes of cells and led to the expansion of cancers [21,22], while other lncRNAs suppressed the processes of cells and inhibited the deterioration of diseases [23–25]. The contrasting roles of lncRNAs suggest that lncRNAs have different functions in the processes of different diseases. Therefore, understanding the roles of lncRNAs in the pathogenesis of hypertrophic scar may bear clinical significance for the diagnosis and treatment of hypertrophic scar.

In this study, we assessed the expression of lncRNA FPASL in HS tissues and HS primary fibroblasts. The results indicated that lncRNA FPASL was considerably downregulated in HS tissues and HS primary fibroblasts. CCK-8 and colony formation assays demonstrated that FPASL overexpression inhibited fibroblast proliferation, and flow cytometric analysis indicated that FPASL overexpression induced G0/G1 phase cell cycle arrest in fibroblasts. These results suggested that lncRNA FPASL suppressed fibroblast proliferation in hypertrophic scar.

Similar to protein-coding genes, lncRNA expression is affected by a variety of factors, such as genetic changes, sex and the natural/social environment [26–28]. Growing evidence suggested that aberrant epigenetic regulations, including acetylation [29], methylation [30], phosphorylation [31], SUMOylation [32] and ubiquitination [33], strongly contribute to the expression and function of lncRNAs [14]. We found many differentially expressed lncRNAs by lncRNA microarray in HS and NS in the present study. GO annotation enrichment analysis revealed that the differentially expressed mRNAs in HS compared to NS were correlated with methylation and methyltransferase activity, which suggested that aberrant FPASL may be caused by methylation. DNA methylation is an epigenetic regulation that mostly occurs in the CpG islands [34]. In this study, two CpG islands were identified in the FPASL promoter, and the DNA methylation inhibitor AZC improved FPASL expression in fibroblasts. This could explain the downregulated FPASL in hypertrophic scar. Consequently, we further measured the DNA methylation level of FPASL in primary fibroblasts from NS and HS tissues, and found that the DNA methylation level of FPASL was increased in HSFBs.

DNA methylation is catalyzed by DNA methyltransferases, including DNA methyltransferase 1 (DNMT1), DNA methyltransferase 3a (DNMT3a) and DNA methyltransferase 3b (DNMT3b) [35]. Our results showed that DNMT3b is the crucial player in the expression of FPASL. There is a series of events that occur when the gene is methylated or unmethylated. DNMT1, DNMT3a and

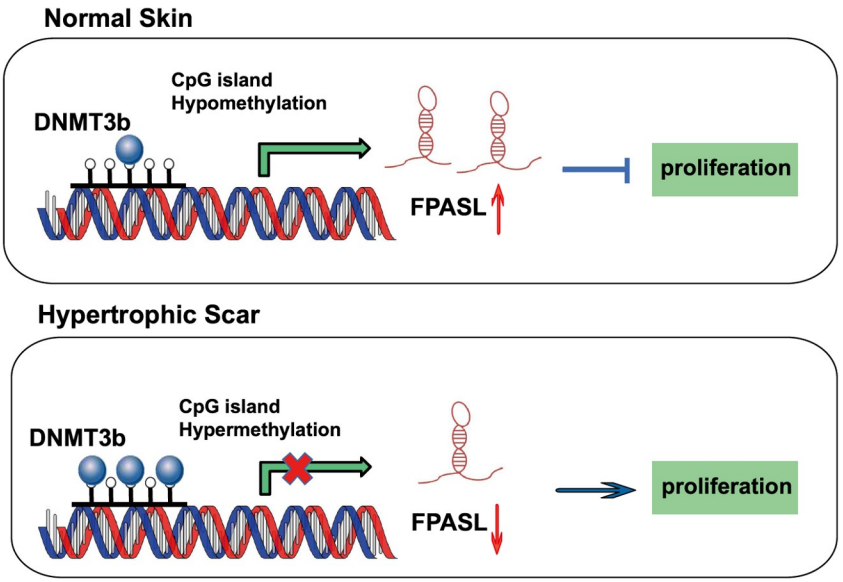

Figure 5. Schematic diagram showing DNMT3b-mediated inhibition of lncRNA FPASL in promoting fibroblast proliferation in hypertrophic scar
The lncRNA FPASL suppresses fibroblast proliferation in hypertrophic scar. Mechanistically, DNMT3b inhibits lncRNA FPASL expression by mediating its promoter hypermethylation in hypertrophic scar.

DNMT3b participate in DNA methylation to regulate normal biological functions, such as cell proliferation, gene transcription and cancer development [36,37]. DNMT1-mediated FOXO3a promoter hypermethylation leads to downregulation of FOXO3a expression, which promotes breast cancer stem cell (BCSC) properties and tumorigenicity in breast cancer [12]. DNA methylation-regulated lncRNA SNHG12 promotes temozolomide resistance in glioblastoma [14]. In this study, we found that DNMT3b mediates FPASL-regulated fibroblast proliferation and cell cycle progression in hypertrophic scar.

In summary, we demonstrated that DNMT3b inhibits FPASL expression, which contributes to fibroblast proliferation and cell cycle progression in hypertrophic scar (Figure 5), and that targeting this process may be a potential therapeutic strategy for hypertrophic scar in future clinical studies.

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